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Previews

Probing Functional Tyrosines

Steven H.L. Verhelst[1,*]
[1]Lehrstuhl für Chemie der Biopolymere, Technische Universität München,Weihenstephaner Berg 3, 85354 Freising, Germany
*Correspondence: verhelst@wzw.tum.de
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Reactivity probes are small molecules that contain a reactive electrophile and a purification handle, and are used to identify hyperreactive amino acid residues within a whole proteome. In this issue of *Chemistry & Biology*, Gu et al. use sulfonyl fluoride derivatives for the identification of functional tyrosine residues in different classes of glutathione S-transferases.

With continuous advances in mass spectrometry, the field of proteomics has matured and is now able to detect thousands of proteins from ever decreasing amounts of cells or tissue material (Cox and Mann, 2011). While classical shotgun proteomics catalogs the abundance of proteins, chemical proteomics uses small molecule probes to fractionate the proteome according to a specific trait. For example, activity-based probes (ABPs) allow a readout of the active site availability of diverse enzyme classes. By means of an electrophilic "warhead," ABPs covalently react with active site nucleophiles in active enzymes but not in zymogens or inhibitor-bound forms. The probe selectivity can be fine-tuned by optimizing the reactivity of the warhead and the structure of the rest of the probe (Figure 1A) (Haedke et al., 2013). While high ABP selectivity is required for some applications like imaging, broad spectrum ABPs are invaluable for profiling a specific family of enzymes.

The concept of reactivity probes (RPs) has recently been introduced into chemical proteomics (Weerapana et al., 2008). RPs only contain a reactive electrophile and a chemical handle for purification, such as a biotin or an alkyne (Figure 1A). When used at low enough concentration, RPs allow the identification of hyperreactive amino acid residues; for example, iodoacetamide probes (Figure 1B). At high concentrations, iodoacetamide derivatives react with all cysteines in a proteome. However, low concentrations allow the identification of functional cysteine hot spots, including active site residues, redox-active disulfides, or sites for post-translational modification (Weerapana et al., 2010).

In this issue of *Chemistry and Biology*, Gu et al. (2013) describe the use of sulfonyl fluoride (SF) derivatives as RPs (Figure 1B). The SF probes are based on 4-(2-aminoethyl)benzenesulfonyl fluoride (AEBSF) and were originally designed to detect serine proteases (Shannon et al., 2012). However, experiments by Gu et al. (2013) on an *Arabidopsis thaliana* leaf proteome revealed that one of the consistently labeled protein bands contained three different glutathione S-transferases (GSTs). For one of the GSTs, a modified peptide was detected, and by careful inspection of the tandem MS data, the modification was mapped to a specific tyrosine residue. Similar experiments on mouse proteomes exposed to SF RPs showed a different labeling pattern than in plant proteomes, but the experiments also revealed the covalent modification of GSTs. In this case, modified peptides were found from three different classes of GSTs: Mu, Omega, and Pi. The modifications were again found on tyrosine residues. Remarkably, these tyrosine residues are not conserved in the GST primary sequences. To further clarify the labeling, the authors made an overlay of the four GSTs of which the labeling site was identified: a crystal structure of the mouse Pi GST and homology models for the mouse Mu and Omega GSTs and the *Arabidopsis* Tau GST. Although the sequence identity of these proteins is low, the overall three-dimensional (3D) structures appear to be quite similar (Figure 2A). Even more striking were the positions of the labeled tyrosine residues: they all cluster around the xenobiotic binding site (H-site), opposite to the glutathione binding site (G-site) (Figure 2B).

Next, the authors took a look at a *Schistosoma japonicum* GST (SjGST) that is widely used as a fusion tag for purification of recombinant proteins. SjGST was also labeled by the SF probe, and examination of a crystal structure showed that tyrosine Y111 is located at an analogous position close to the H-site as in the other GST structures. Mutation of this tyrosine to a phenylalanine indeed prevented labeling. More importantly,

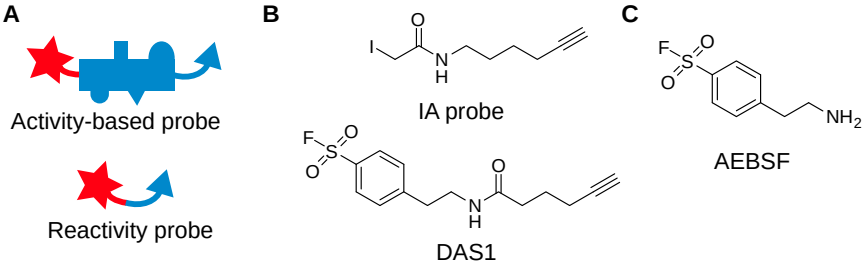

Figure 1. Covalent Chemical Probes
(A) Activity-based probes can be tuned in their selectivity by means of a warhead and an additional recognition element (blue) that fits to the target enzyme's active site. The tag (red) can subsequently be used for visualization or enrichment. Reactivity probes only contain a reactive electrophile for covalent attachment to hyperreactive nucleophilic residues within the proteome.
(B) Examples of two reactivity probes: a iodoacetamide-based (IA) probe (Weerapana et al., 2010), and a sulfonyl fluoride (DAS1) probe (Gu et al., 2013).
(C) The serine protease inhibitor AEBSF on which the design by Gu et al. (2013) was based.

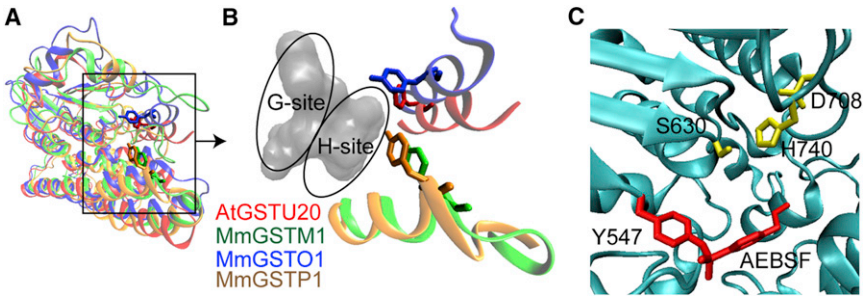

Figure 2. Sulfonyl Fluorides Bind to Tyrosine Functional Hot Spots
(A) An overlay of a crystal structure and homology models of GSTs from four different classes. Despite the low sequence identity, the 3D structures have a good overlap.
(B) A close-up view of the tyrosine residues labeled by the SF probe (Y208, red; Y116, green; Y228, blue; Y108, orange). All tyrosines are in close proximity to the xenobiotic binding site (H-site) and further away from the glutathione binding site (G-site).
(C) A close-up view of the active site of DPPIV, with AEBSF bound to Y547 (both in red). The catalytic triad S630, H740, and D708 are depicted in yellow (Protein Data Bank ID code 2AJC).

the mutant displayed a severely reduced catalytic efficiency, demonstrating the functional importance of this tyrosine residue. Overall, the results by Gu et al. (2013) highlight that SFs are suitable RPs that can be used to reveal tyrosine residues as functional hot spots in proteins.

As mentioned, the basic structure of RPs developed by Gu et al. (2013) is AEBSF (Figure 1C), a commonly used serine protease inhibitor also marketed as Pefabloc. As described here and elsewhere, its reactivity is not restricted to serine residues. This was observed even in the case of serine proteases themselves, as illustrated by a crystal structure of the serine protease DPPIV in complex with AEBSF. In this complex, the SF can also be seen bound to a tyrosine residue (Engel et al., 2006). Interestingly, the particular tyrosine is part of the oxyanion hole, and hence, also represents a functional hot spot (Figure 2C). Evidently, the usage of AEBSF as a selective inhibitor for serine proteases should be discouraged, but the molecule has gained a function as a scaffold for RPs. Since Gu et al. (2013) identify tyrosines in many more proteins besides GSTs, the sulfonyl fluoride probes hold great promise for the future identification of functional tyrosines in other protein families.

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Photoswitchable Protein Degradation: A Generalizable Control Module for Cellular Function?

Evan Mills[1,*] and Kevin Truong[1,2,*]
[1]Institute of Biomaterials and Biomedical Engineering, University of Toronto, 164 College Street, Toronto, Ontario, M5S 3G9, Canada
[2]Edward S. Rogers Sr. Department of Electrical and Computer Engineering, University of Toronto, 10 King's College Circle, Toronto, Ontario, M5S 3G4, Canada
*Correspondence: e.mills@mail.utoronto.ca (E.M.), kevin.truong@utoronto.ca (K.T.)
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In this issue of *Chemistry & Biology*, Renicke et al. report a photosensitive degron (psd) consisting of the LOV2 domain fused to a protein degradation sequence. This design enabled light-dependent protein degradation in yeast. When psd was fused to cell-cycle-dependent proteins, it controlled cell cycle by light with spatiotemporal precision.

The development of real-time, protein-based tools to control cellular functions promises everything from new assays for basic research to gadgets to be built into sophisticated synthetic biomachines. Recent reports have highlighted tools to target key nodes in protein networks regulating gene expression (Levskaya et al., 2005; Strickland et al., 2008, 2010), cell

